REMARKS

Claims 1-4, 6, 12-14, 17-18, and 26-27 are pending in the application. Claims 12-14 were previously withdrawn without prejudice. Claims 5, 7-11, 15-16, and 19-25 were previously cancelled. In the instant response, claim 1 has been amended. Support for the amendment to claim 1 can be found throughout the specification, for example on page 10, third full paragraph. Upon entry of the instant amendment, claims 1-4, 6, 17-18, and 26-27 will be before the Examiner for consideration.

Withdrawal of objections and/or rejections

Applicant thanks the Examiner for withdrawal of all of the previous rejections for clarity, enablement, and obviousness in the instant case.

Rejections Under 35 U.S.C. § 103

The Office Action has newly rejected claims 1-4, 6, 17-18 and 26-27 under 35 U.S.C. 103(a) for allegedly being unpatentable over Kosaka et al. (*Exp Cell Res* 245: 245-251, 1998), and Tropepe et al. (*Science* 287: 2032-2036, 2000), in view of Pardo et al. (*Brain Res* 818: 84-95, 1999), and further in view of Lee et al (*Theriogenology* 44: 71-83, 1995) and Samarut et al., (US Patent number 6,500,668, dated 31 December 2002), as evidenced by Reynolds et al. (*Science* 255: 1707-1710, 1992) and Kitchens et al. (*J. Neurobiol* 25: 797-807, 1994).

The Office Action states that the claims are drawn to a method for producing myocardial tissue cells comprising: (i) obtaining iris pigment epithelial cells and dissociating the isolated cells; (ii) culturing epithelial cells by floated coagulated mass in serum free media with N2 supplement and obtaining pluripotent stem cells (claim 1-3), wherein the stem cells are Oct-3/4 positive (claim 4); (iii) obtaining myocardial cells from the pluripotent stem cells by culturing the cells in avian and fetal calf serum containing media, wherein the medium also contains a growth factor (FGF2 and EGF). The claims are said to further recite the extirpation of iris tissue by excising the tissue, treating with enzyme, and restoring the tissue in medium containing fetal calf serum (claim 6, 26-27).

The claims are also said to recite testing for a myocardial cell specific gene, for example myosin (claims 17, 18).

Kosaka is alleged to teach the removal of eyeballs from 1 day old (postnatal chicken, followed by incision around the iris, incubating the tissue in dispase solution and thereafter in EDTA (page 246, col 1, para 3), isolating the pigmented epithelial cells, culturing the cells in Eagle's MEM (EMEM) medium containing fetal bovine serum, dissociating the cells into a single cell suspension after treatment with 0.1% trypsin in PBS, and growing the iris derived pigmented epithelial cells in culture for 18 days before reaching confluency. The cells are harvested and transdifferentiated to lens tissue using EMEM medium with serum and FGF (page 246, column 1, "Procedure for cell culture"; page 248, col 1, para 2).

Kosaka does not teach culturing by the floated coagulation method.

This deficiency is stated to be overcome by the teachings of Tropepe which allegedly teaches the formation of free-floating PCM spheres by the method of Reynolds who is alleged to teach culturing in serum-free culture medium and a combination of hormones and salts that is alleged to be equivalent to N2 supplement.

Kosaka et al. and Tropepe et al. do not teach rotation of the IPE cells.

This deficiency is said to be overcome by Pardo who allegedly teaches aggregating brain cell cultures as a useful *in vitro* model for brain ischemia.

Lee is relied upon to teach that the addition of FGF and EGF have a synergistic effect on increasing the number of blastocysts and the development of embryo *in vitro*.

Finally, Samarut is relied upon to teach the culture of bird embryonic stem cells using a culture medium comprising fetal bovine serum and chicken.

The Office Action relies on seven references to make the rejection of the instant claims. Applicant submits that there can be no reasonable expectation that the combination of references would provide a method for producing myocardial cells from iris pigment epithelial cells as claimed, and that the combination could only be made with impermissible hindsight.

In the Office Action of February 9, 2009 discussed the predictability in the selection of growth factors to promote primary cell growth and differentiation (page 7). The comments are reproduced, in part, below:

> It is well-known in the art that endogenous and exogenous factors govern the expansion, maintenance and differentiation of stem cells in vitro, a prime one being the cultivation condition. For example, Reynolds et al. (Sc. 255: 1707-1710, 1992; page 1707, col 2, para 1) teach that cell division and proliferation to form spheres **was only achieved with EGF, however, was not mimicked by NGF or bFGF**. Mokry et al teach that in case of adherent cultures of neural stem cells, various factors affect cell differentiation and the ratio of the resulting cell types. Modifications in culture conditions influencing cell differentiation include medium constituents like serum, growth factors, hormones, differentiation factors, etc. **Mokry et al provide a cautionary note stating that "a change in cultivation condition resulted in cell death that reduced the numbers of cells that differentiated"** (*Acta Med* 50: 35-41, 2007; page 39, para 2). However, the relevant literature, does not teach that iris pigment epithelial cells can be induced to differentiate into myocardial cells by culturing the IPE cells under any culture condition as broadly claimed. **The skilled artisan will not be able to make and use the claimed invention, thereby entail innumerable trials and errors leading to undue experimentation.**

Applicant submits that the statement in the prior Office Action that "the relevant literature, does not teach that iris pigment epithelial cells can be induced to differentiate into myocardial cells by culturing the IPE cells under any culture condition as broadly claimed" demonstrates that provided with the broad teachings of cell culture available in the art, one of skill in the art could not select and combine the references as suggested in the Office Action in the absence of the disclosure of the instant application. Although all of the components of the instantly claimed invention may have been known in the art at the time of filing of the instant application, the invention relies on the specific combination of timing and growth conditions to provide the invention. The specifically claimed timing and growth conditions claimed cannot be obvious in view of the cited art.

Applicant submits that the rejection based on the seven references is not considering the unpredictability in the field as set forth in the earlier Office Action. If one of skill in the art could not practice the method of the invention as it was previously claimed without undue experimentation, one of skill in the art could not combine the

references to provide the instantly claimed invention with a reasonable expectation of success without the use of impermissible hindsight.

The references cited in the instant Office Action teach the difficulty of culturing primary cells. For example, Therefore, Kosaka teaches that:

1. Culturing primary cells is difficult;

2. Selection of growth conditions and growth media cannot be predicted; and

3. Growth and maintenance of two highly related primary cell cultures, RPE cells and IPE cells, are drastically different, one being highly unpredictable, and one being predictable under at least one set of growth conditions.

Further, it is disclosed in Kosaka that iris pigmented epithelial cell (IPE) culturing methods and a retinal pigment epithelial cell (RPE) culturing methods are substantially different from each other.

Moreover, the specific differentiation inducing conditions are neither disclosed nor suggested in the seven references, Kosaka, Tropepe, Pardo, Lee, Samarut, Reynolds, and Kitchens, which form the basis of the rejection, either alone or in combination with each other. Since none of the cited documents discloses or suggests the specific differentiation inducing conditions now claimed, the presently claimed invention cannot be obvious in view of a combination of the cited documents. The references alone or in any combination with each other cannot teach the instantly claimed method which includes growth of cells using a floated coagulated mass culturing technique in serum free media containing an N2 supplement and at least one of FGF, LIF, or SCF.

Kosaka teaches growth and maintenance of IPE cells in EMEM supplemented with dialyzed FBS, to remove small proteins such as growth factors and glutamine. It would be contrary to the teachings of Kosaka to add at least one of FGF, LIF, or SCF to media supplemented with **dialyzed FBS** which would intentionally have these substances removed.

Tropepe discloses culturing of cells by a neurosphere method, which method is relevant to the floated coagulated mass culturing technique of the invention of the

present application. However, Tropepe uses an isolated pigment cell (PCM) derived from a ciliary margin of a mouse, which is different from the iris pigmented epithelial cells (IPE) that serve as the material in the method of the present invention for producing tissue cells (more specifically, myocardial cells).

Further, Tropepe neither discloses nor suggests that the culturing method provided therein is applicable to culturing of iris pigmented epithelial cells (IPE) which are different cells. Further, Tropepe suggests nothing of using a culture medium containing an N2 supplement and at least one of FGF, LIF, and SCF, as in added in amended claim 1.

Moreover, based on the descriptions in Tropepe, it is considered that a cultured pigment cell, obtained by isolating a pigment cell (PCM) from a ciliary margin of a. mouse and then culturing the pigment cell by the neurosphere method, can only differentiate to cells that are derived from a retinal stem cell.

However, the invention according to the claims of the present application obtains myocardial cells, which are different from retinal cells, from iris pigmented epithelial cells (IPE) taken from an eye of an animal.

Thus, even though Tropepe discloses culturing of cells by the neurosphere method, it is impossible from the description therein to arrive at the instantly claimed method of inducing differentiation to myocardial cells that are a completely different type of cell from the original cell type. Similarly, the other cited references provide no teachings or suggestion that cells from eye could be differentiated into such a substantially different cell type.

None of the cited references discloses nor suggests inducing differentiation of cells derived from any ocular tissue to myocardial cells. Particularly, Lee, Samarut, and Kitches which are newly cited in the instant Office Action only teach the effects of serum or factors that are included in the culture medium under the differentiation conditions in the claims of the present application. They do not teach the full method claimed. Hence, it is impossible to arrive at the instantly claimed invention which includes "obtaining myocardial cells by culturing pluripotent stem cells in a culture medium

containing fetal calf serum, avian serum, EGF, and FGF2 for one to two months", even when the references are considered in combination.

Therefore, the invention as claimed cannot be considered obvious in view of any of the cited references in any combination. Withdrawal of the rejection is respectfully requested.

Conclusion:

In view of the amendments and arguments presented herein, Applicants submit that the claims are in condition for allowance.

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Respectfully submitted,

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